Could Fish Help Us Treat Cancer?: Study of Pore Formation by Piscidin-1

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NIST National Institute of Standards and Technology U.S. Department of Commerce

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CLEMSON UNIVERSITY SOUTH CAROLINA 1889

Antimicrobial Peptides

- Part of the innate immune system of organisms across all classes of life
  - Help fight off diseases, infections
  - Have different mechanisms of action
- Short: 12-50 Amino Acids long
- High AMP content in marine fish
  - Teleost fish (ray finned)
  - **Piscidin-1**

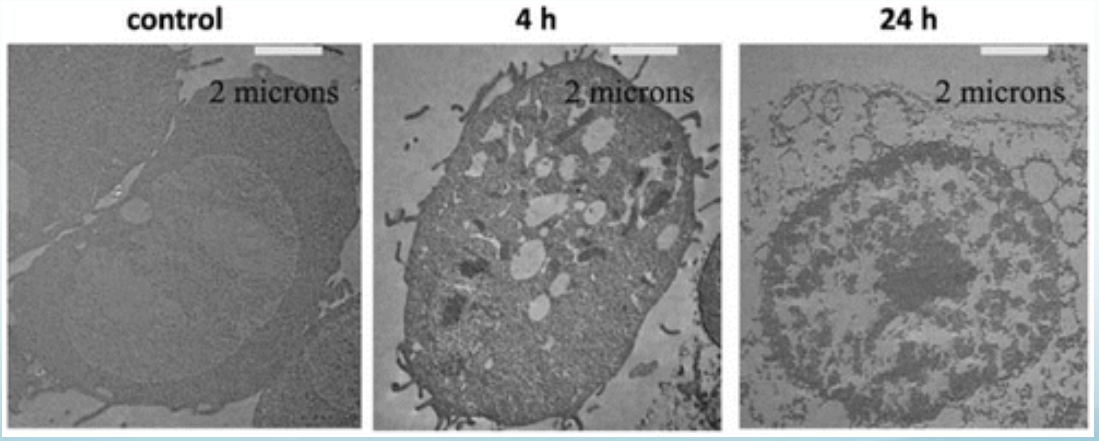
Piscidin-1

- Piscidin-1 (P-1) or Moronecidin
- Found in mast cells of Hybrid Striped Bass
- 22 Amino Acids
  - FFHHIFRGIVHVGKTIHRLVTG
  - MW: 2573 Da
- Unstructured in water
- Alpha helix in membranes
- Amphipathic, cationic (+)
  - Antimicrobial activity

Piscidin-1

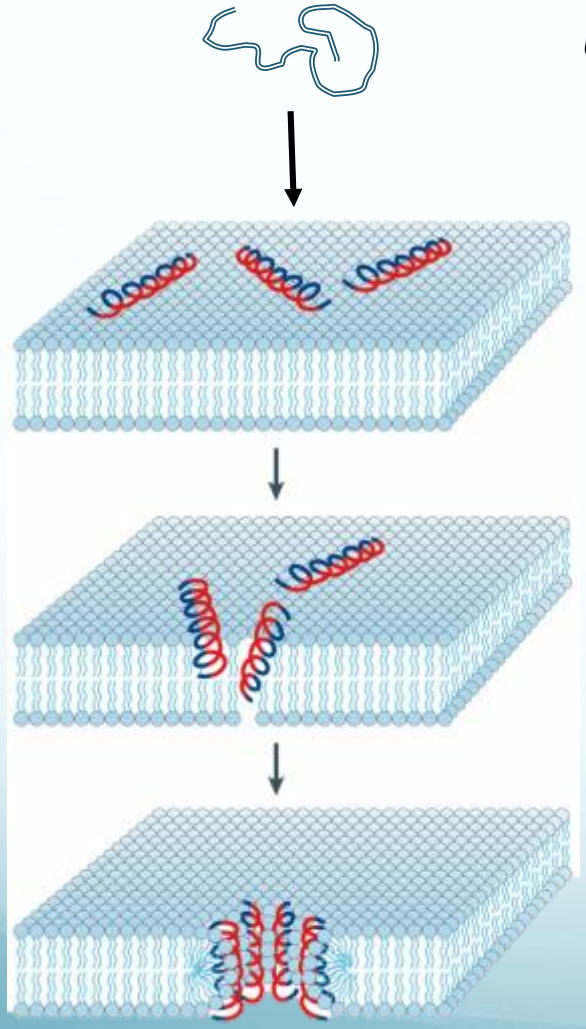
- Antimicrobial Activity
  - Gram-positive and Gram-negative bacteria
  - Dye leakage experiments

  - <u>Some cancer cells</u>
    - Possible therapeutic agent



Lin H –J. et al , Zool. Sci 29 (2012)

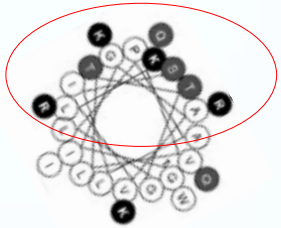
Proposed Mechanism



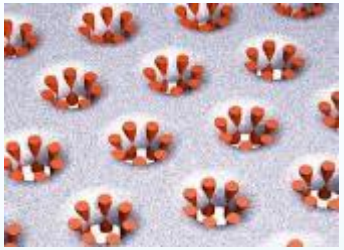
Unstructured in solution

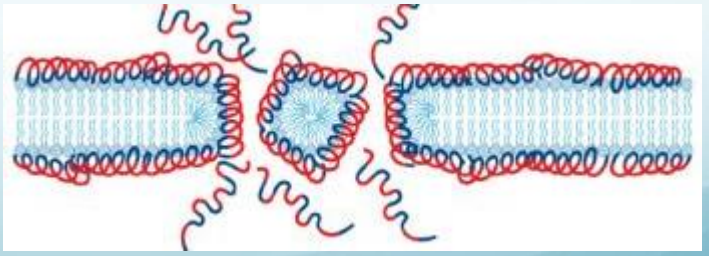
Favorable interaction with the bilayer

Polar side



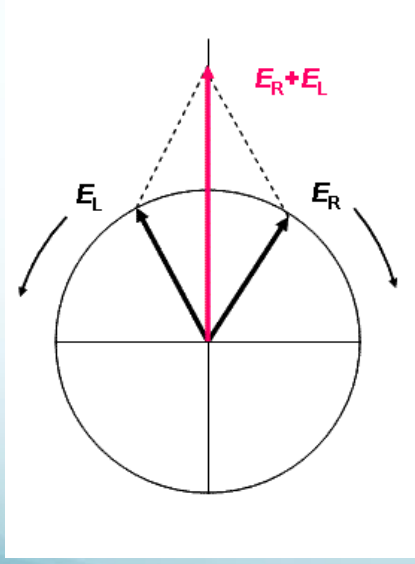
Hydrophobic side



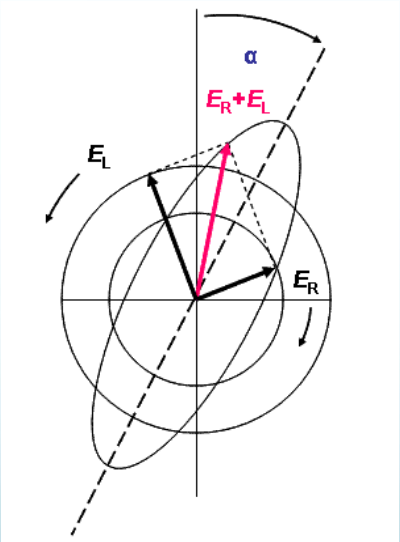


Circular Dichroism (CD)

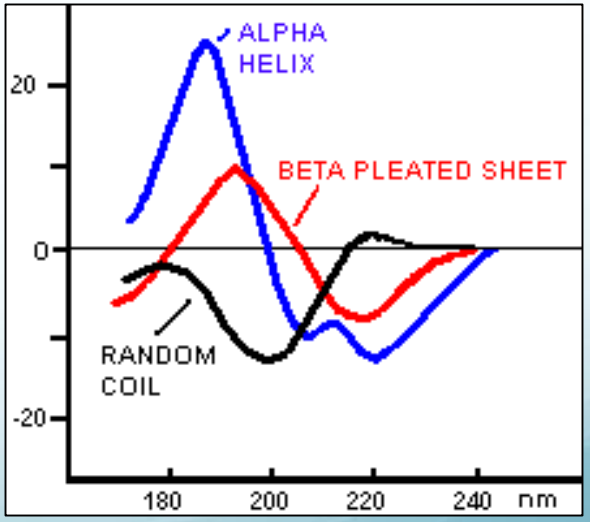
- Different absorbance of right/left polarized light due to asymmetry of molecule
  - CD spectra changes with secondary structure
- IBBR spectropolarimeter



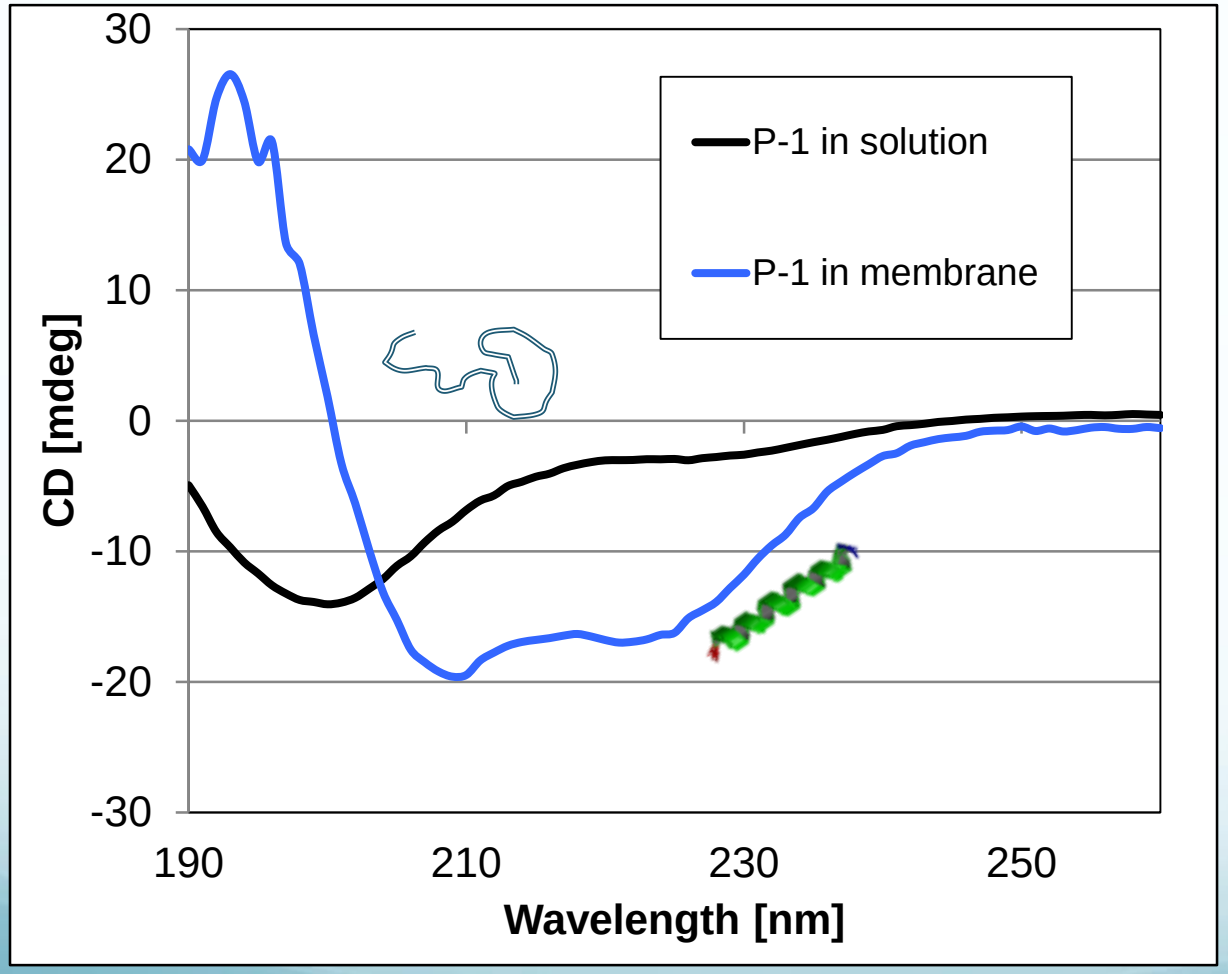
E_0 : plane-polarized beam

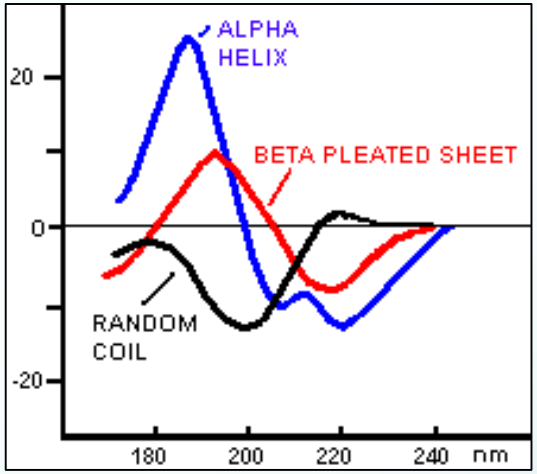


E : elliptically polarized beam



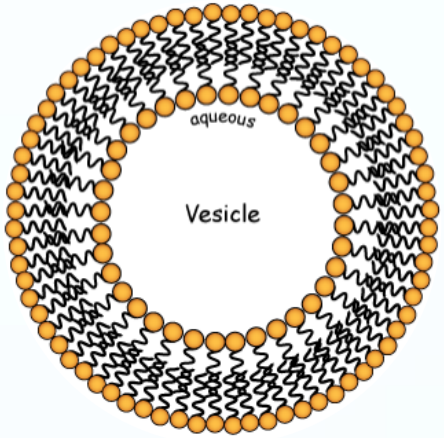
Circular Dichroism (CD)

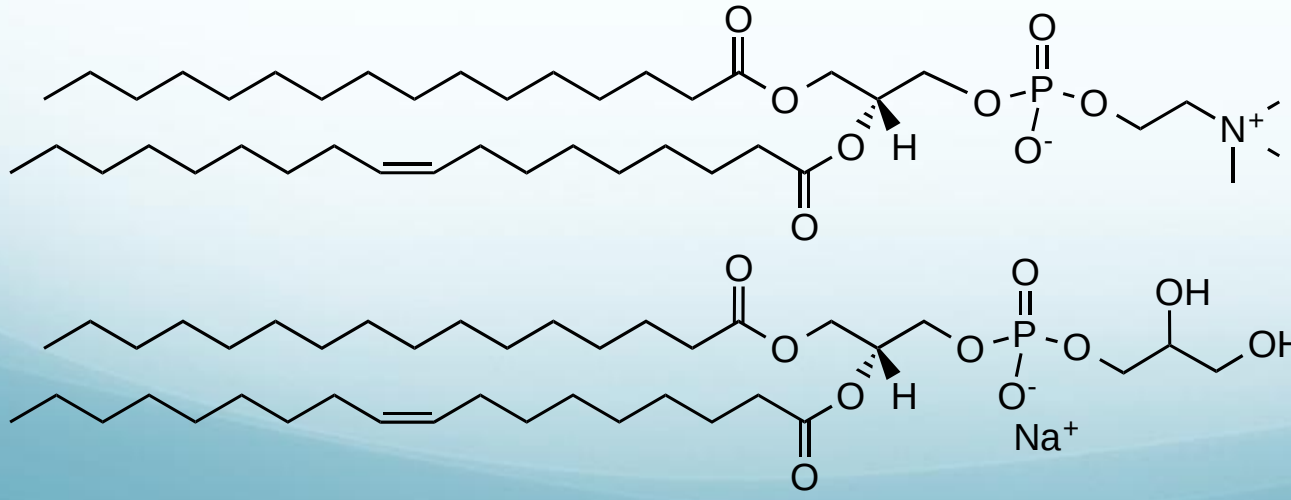




How to study pore formation?

- Vesicles formed from:
  - POPC/POPG (3:1)



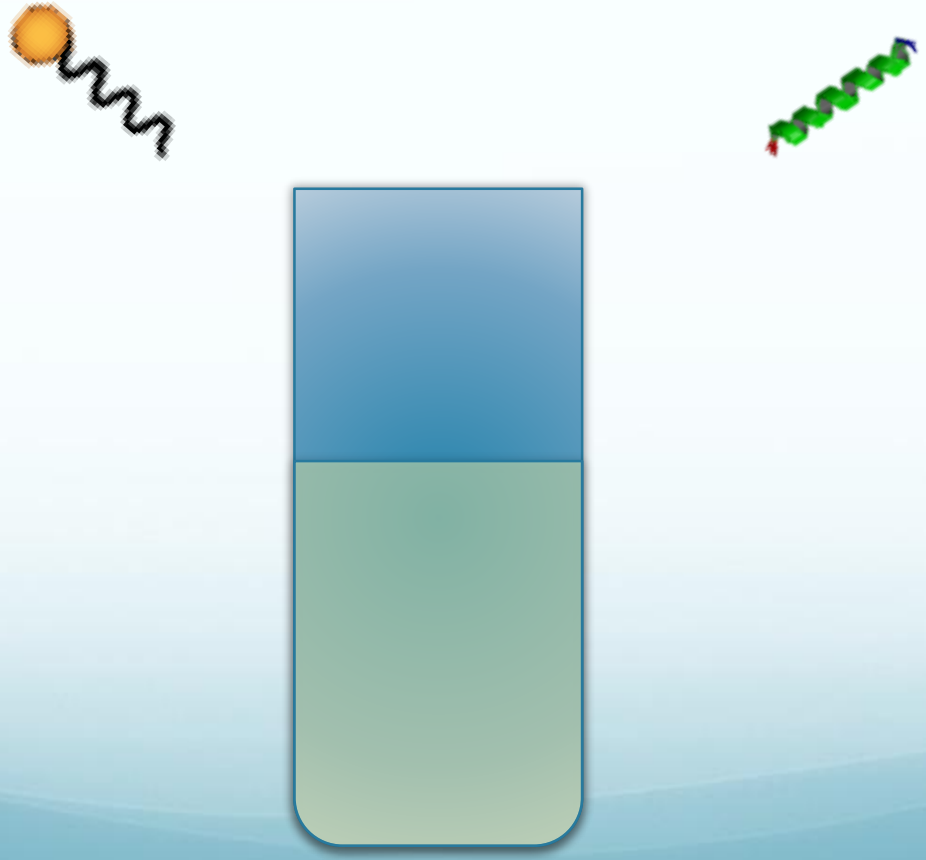


POPC
(no net charge)

POPG
(net (-) charge)

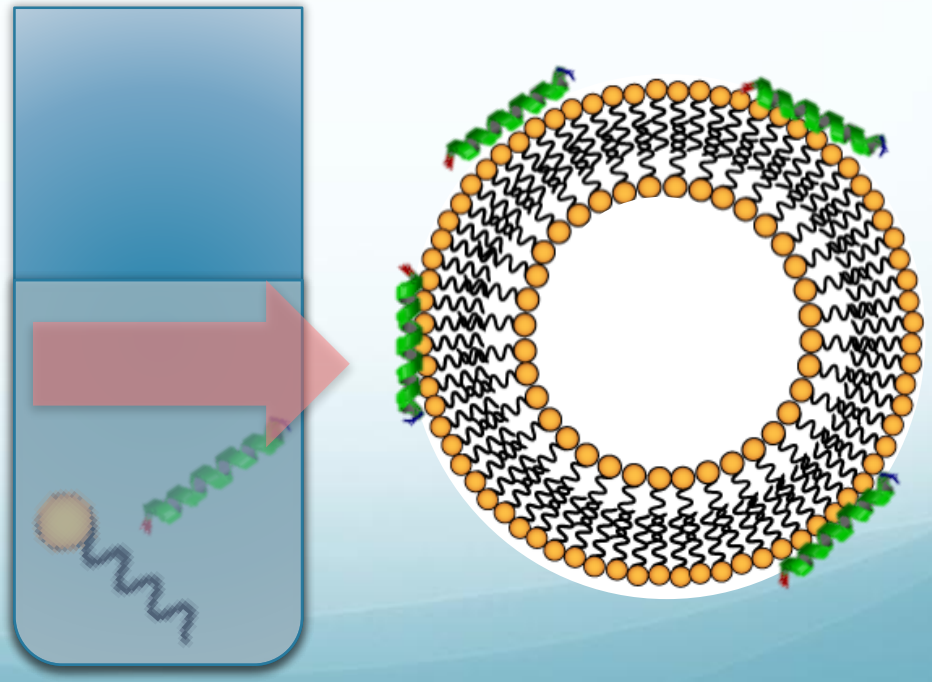
Materials and Methods

- Vesicles formed by:
  - Mixing lipids and peptide in organic solvents



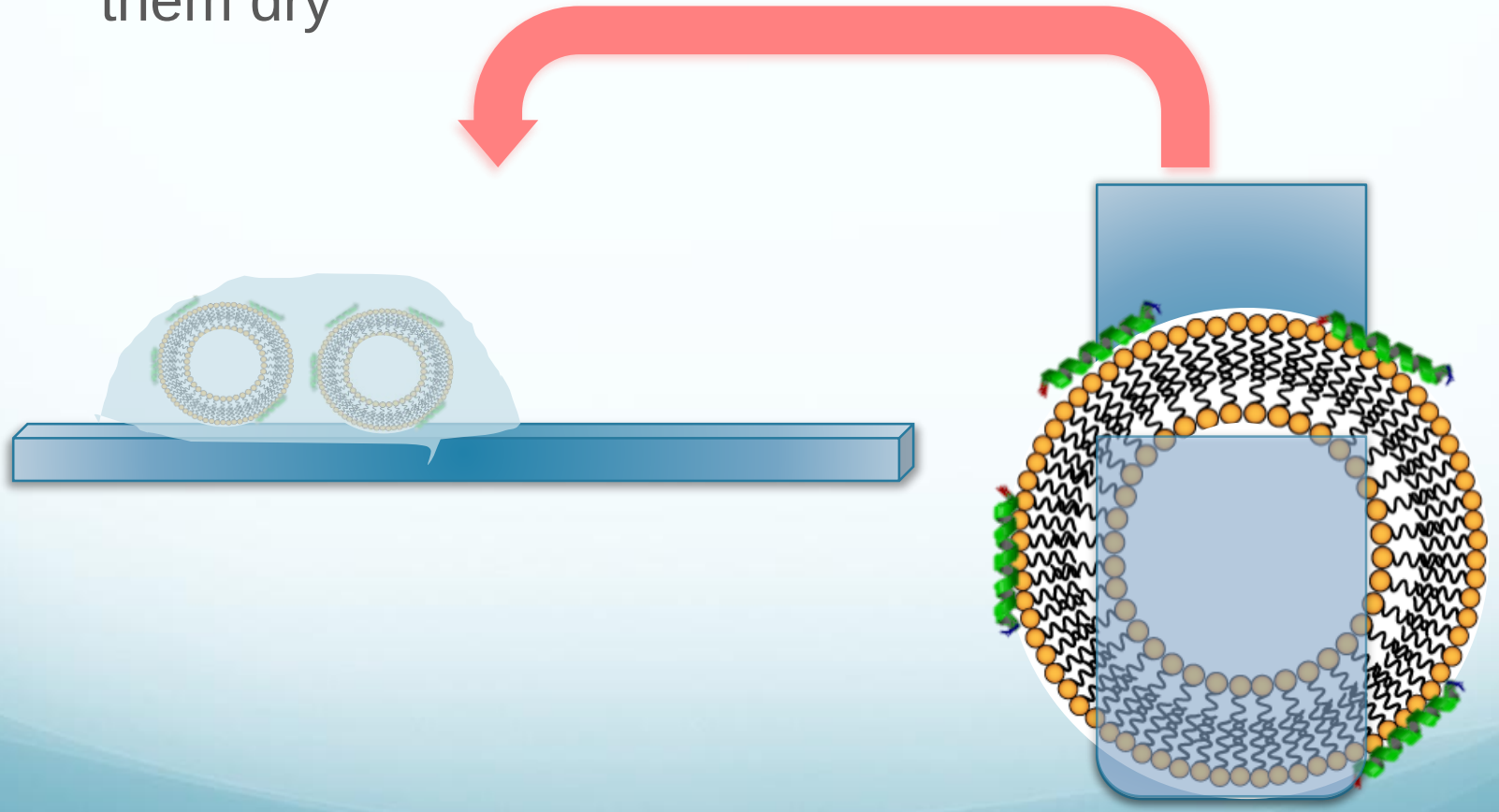
Materials and Methods

- Vesicles formed by:
  - Drying organic solvent and hydrating with H_2O
  - Lipid vesicles form spontaneously



Materials and Methods

- Deposit vesicles with peptide onto a substrate and let them dry



Materials and Methods

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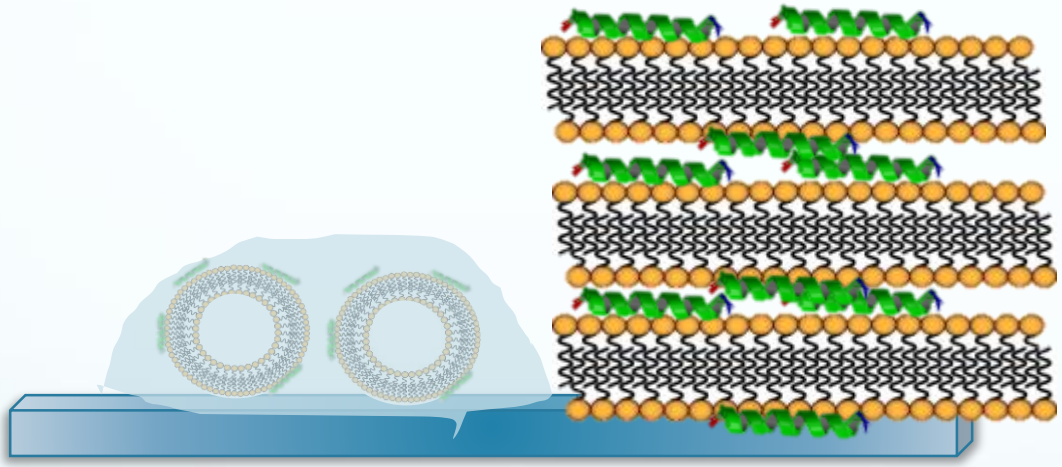
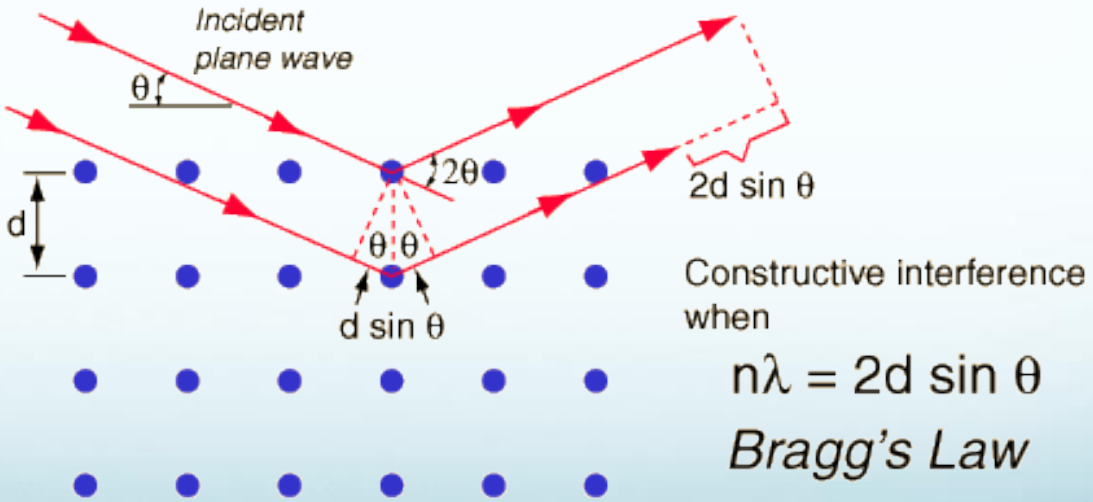


- Liquid crystal structure upon hydration of sample
  - Periodic structure -> diffraction measurements

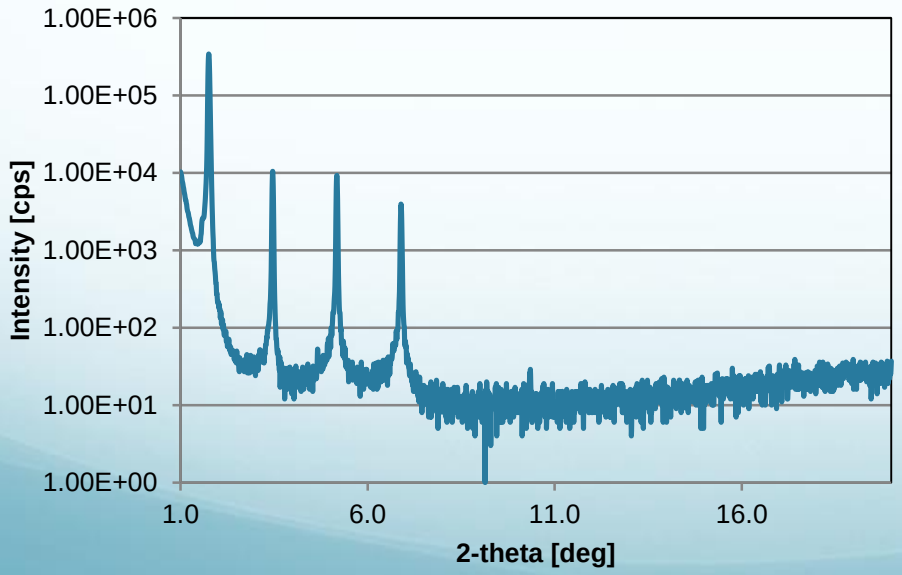
Bragg's Diffraction

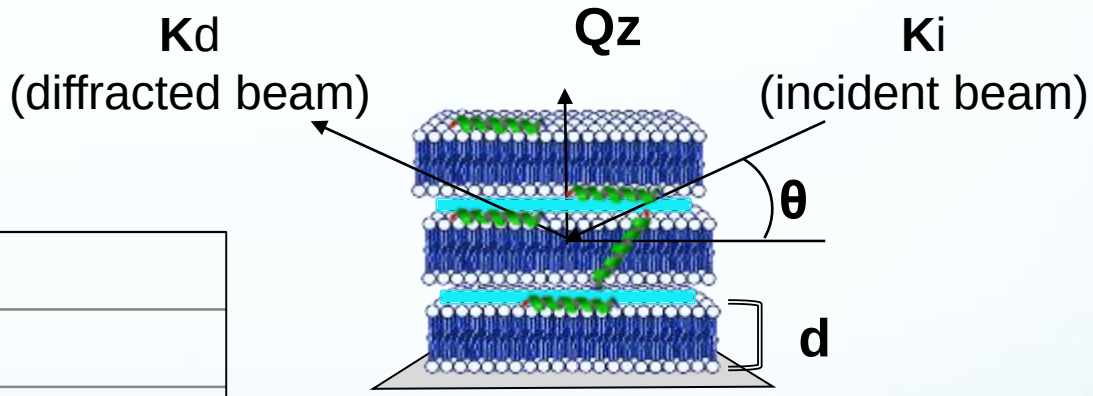
- Electromagnetic beams directed onto lattice, diffracted elastically: diffraction spectra
  - Peaks: constructive interference, repeat distance
  - Intensity: structure factors, sample profile



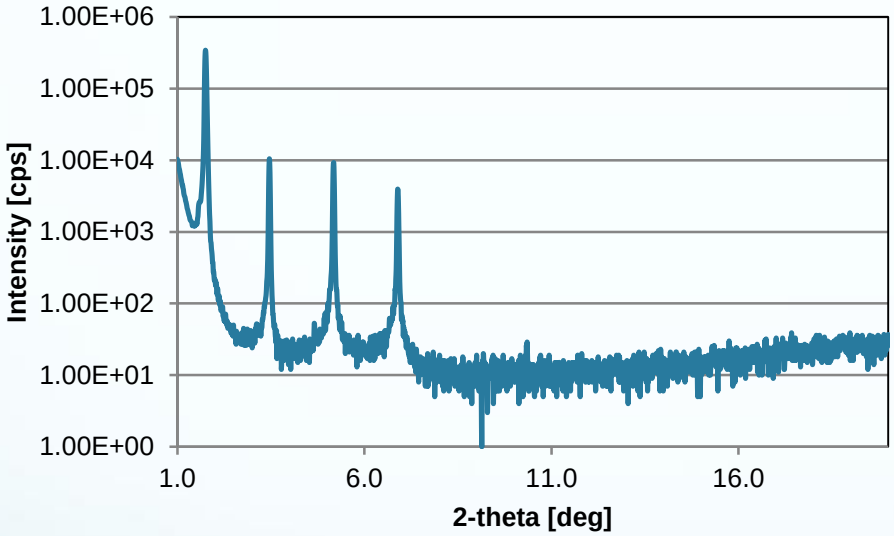
X-ray Diffraction

- Out of Plane XRD
- IBBR X-ray diffractometer

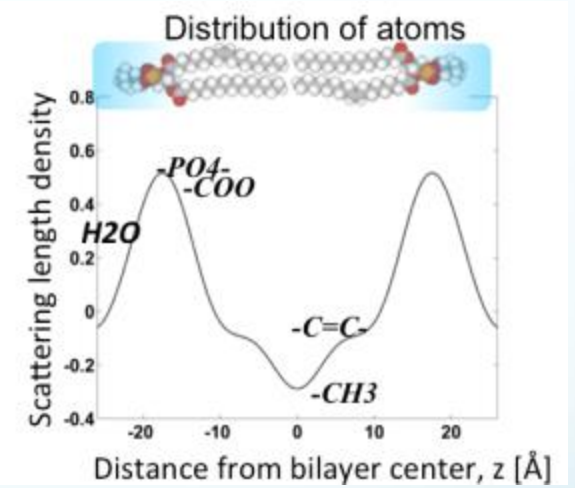




Out of Plane XRD



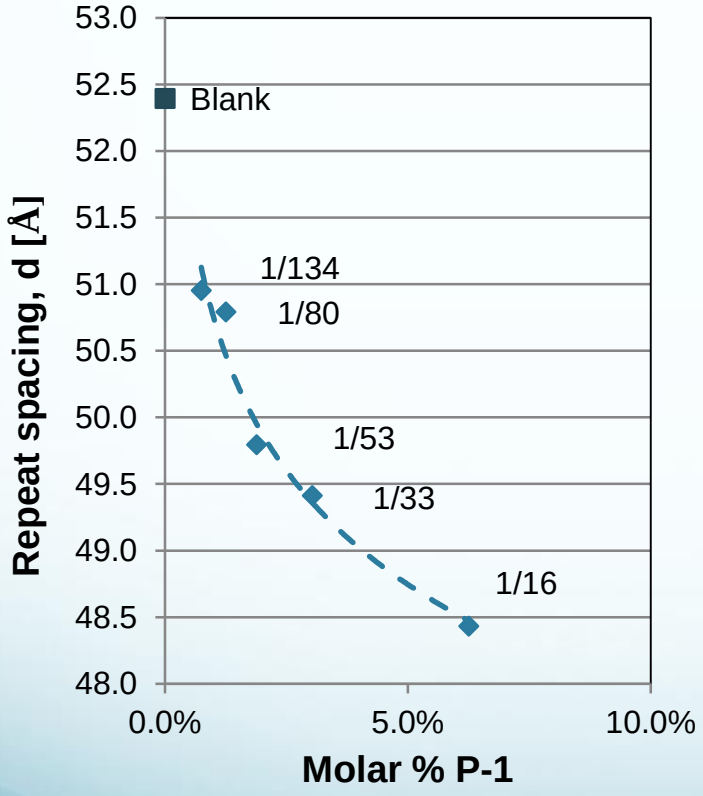
Fourier Analysis

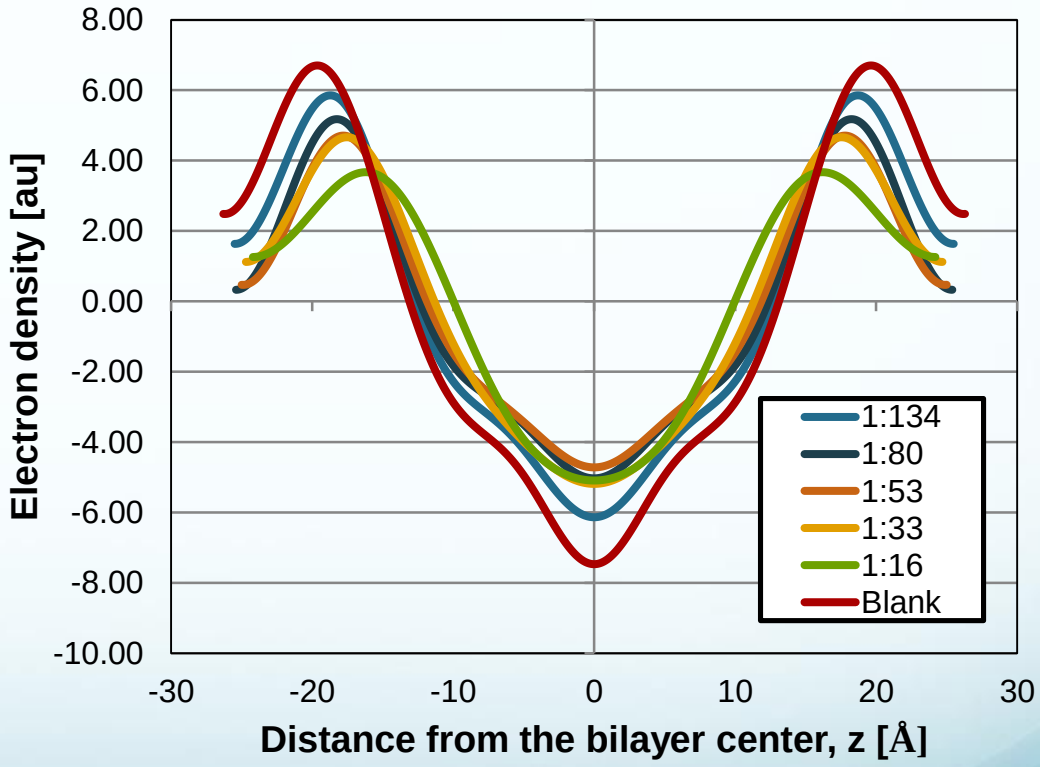


Bragg's law

Bilayer thickness

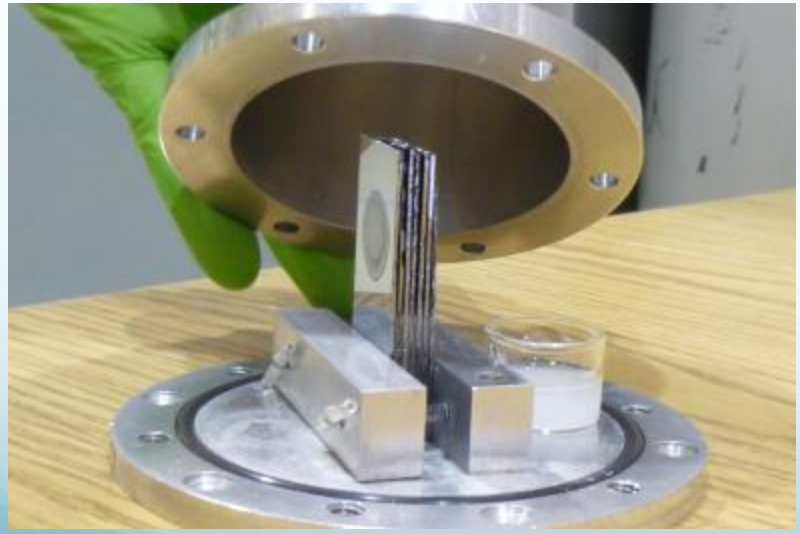
Effects of increasing P-1 content on lipid bilayer

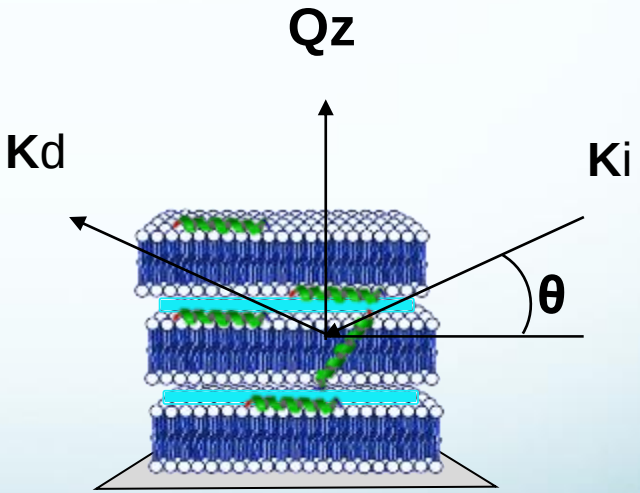




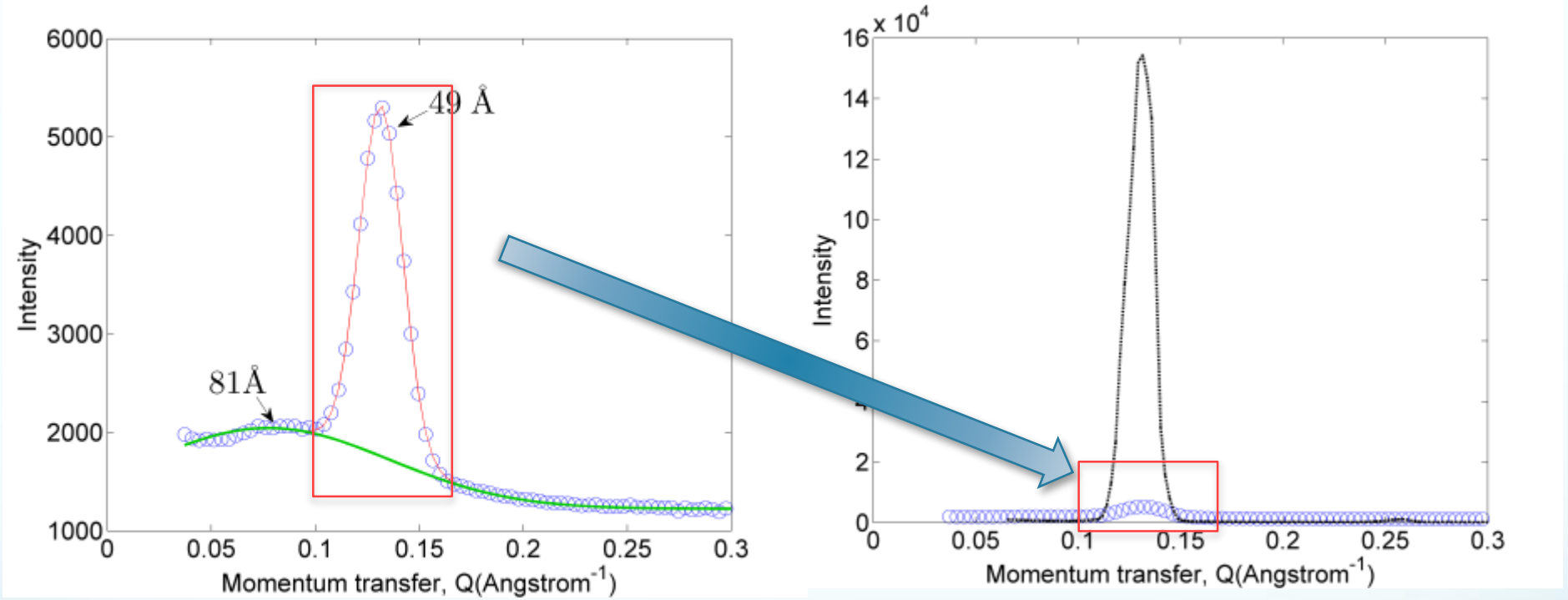
SANS

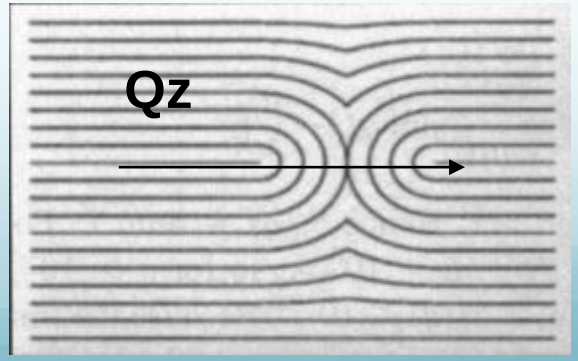
- Momentum transfer vector Q parallel to bilayers
  - Study in-plane features: pores, with D_2O contrast
- NCNR NGB30 SANS





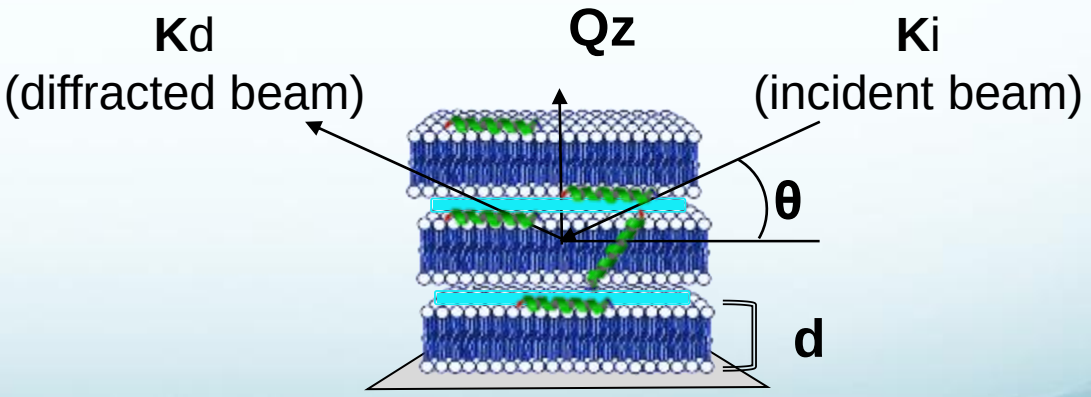
SANS


6000
5000
4000
3000
2000
1000
Intensity
49 Å
81Å
0
0.05
0.1
0.15
0.2
0.25
0.3
Momentum transfer, Q(Angstrom^{-1})
x 10^4
16
14
12
10
8
6
2
0
Intensity
0
0.05
0.1
0.15
0.2
0.25
0.3
Momentum transfer, Q(Angstrom^{-1})

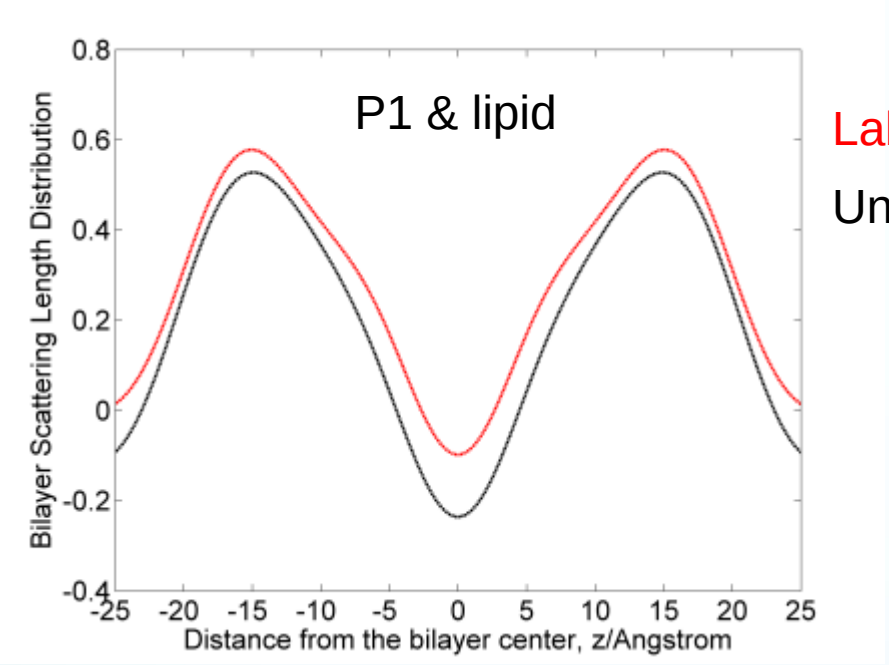

Qz

Neutron Diffraction

- Out of Plane
- Use of contrast (labels, H_2O-D_2O exchange)
- NCNR MAGIK



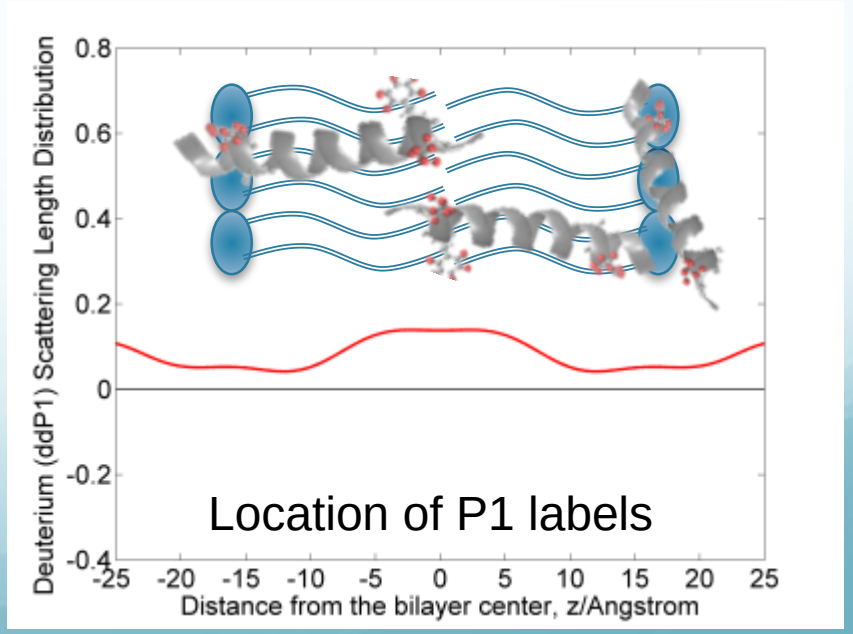
ND: Peptide Distribution



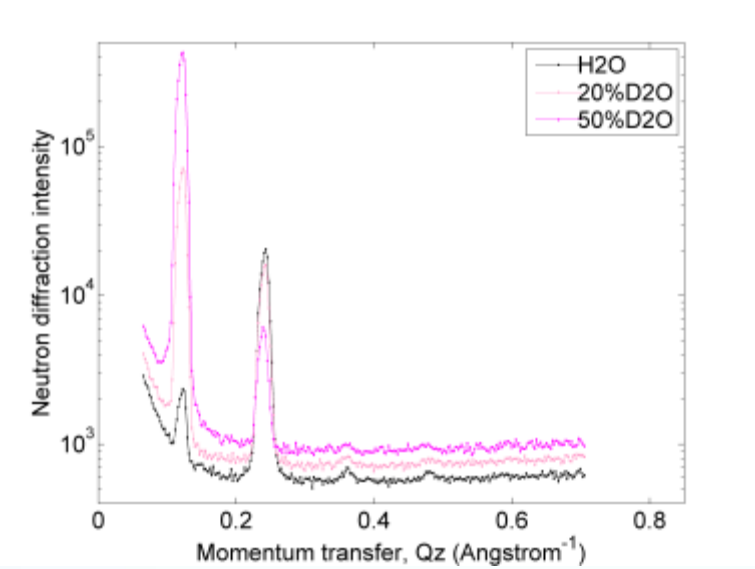
Labeled

Unlabeled

ddP1 FFHHIFRGIVHVGKTIHRLVTG

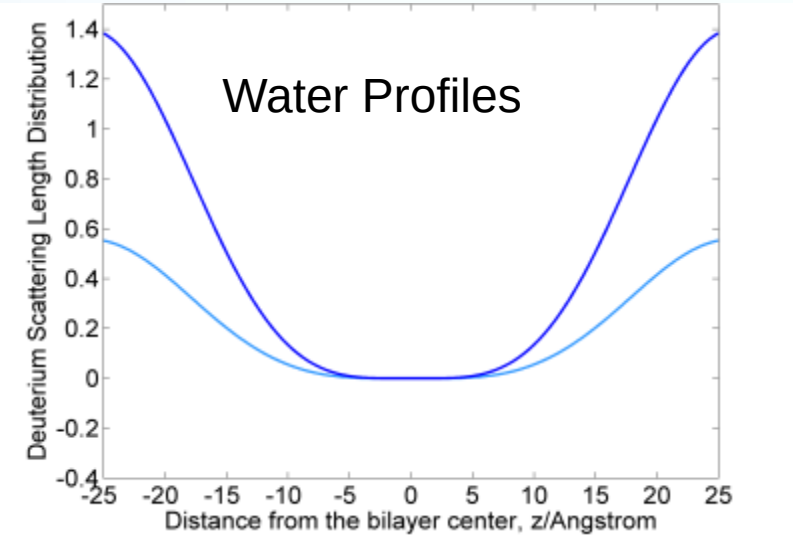


ND: Water Distribution

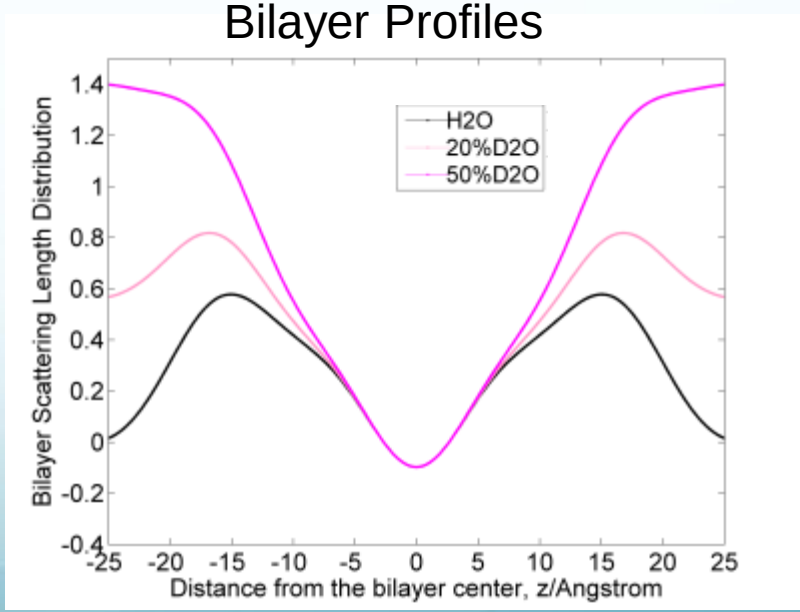


Fourier Analysis

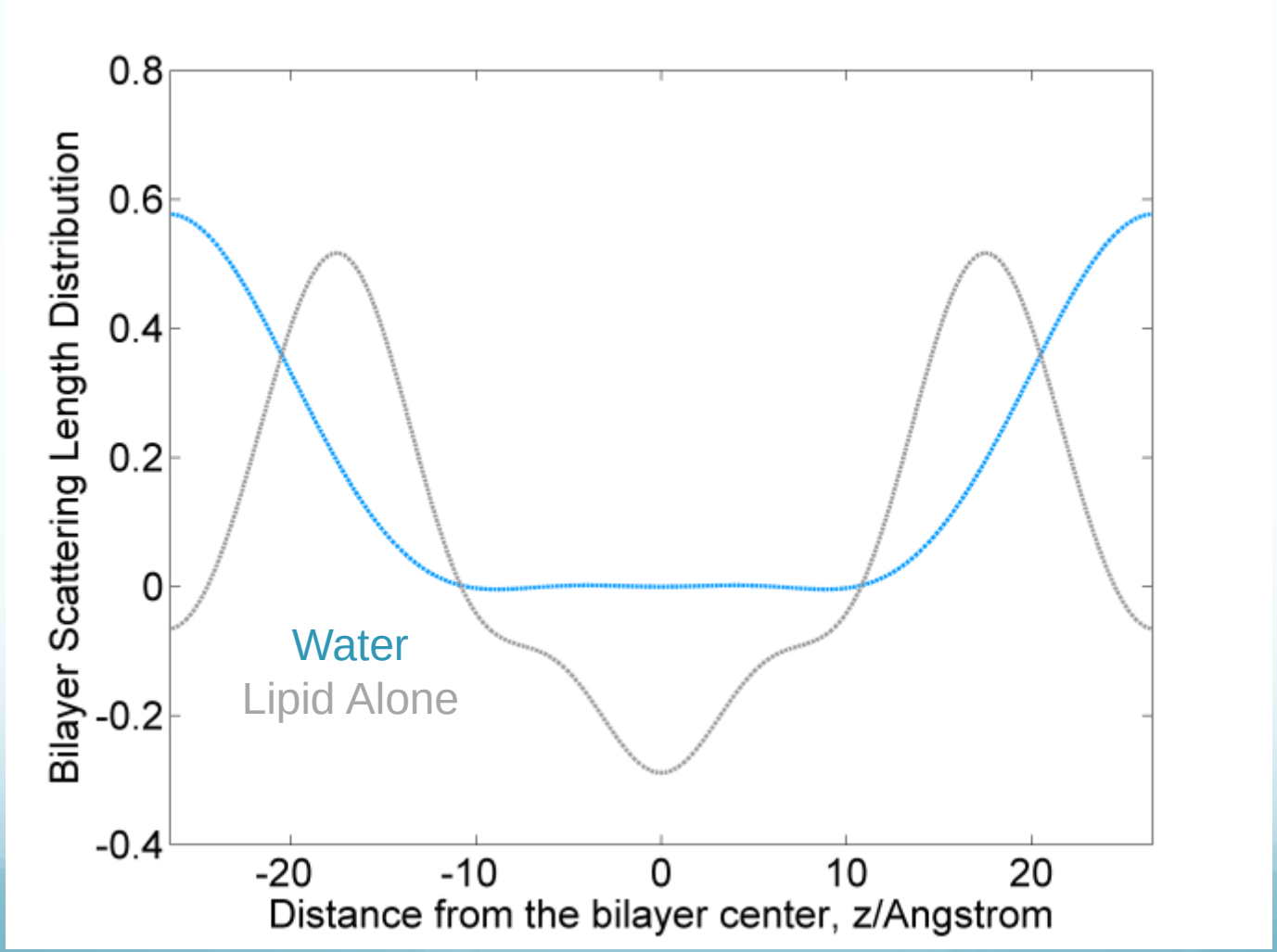
Water Profiles



Bilayer Profiles



Effect of P-1 on Lipid Membrane



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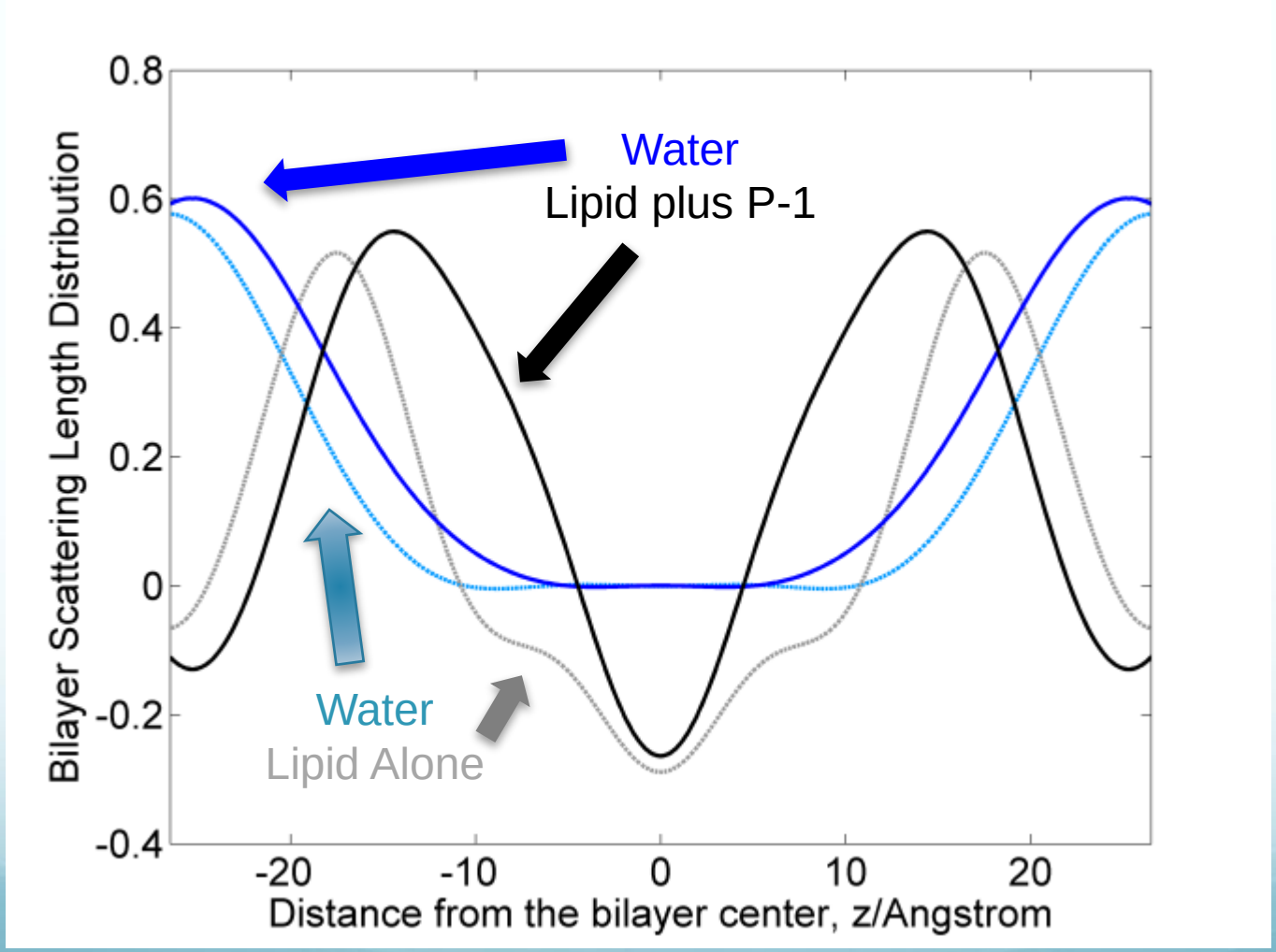
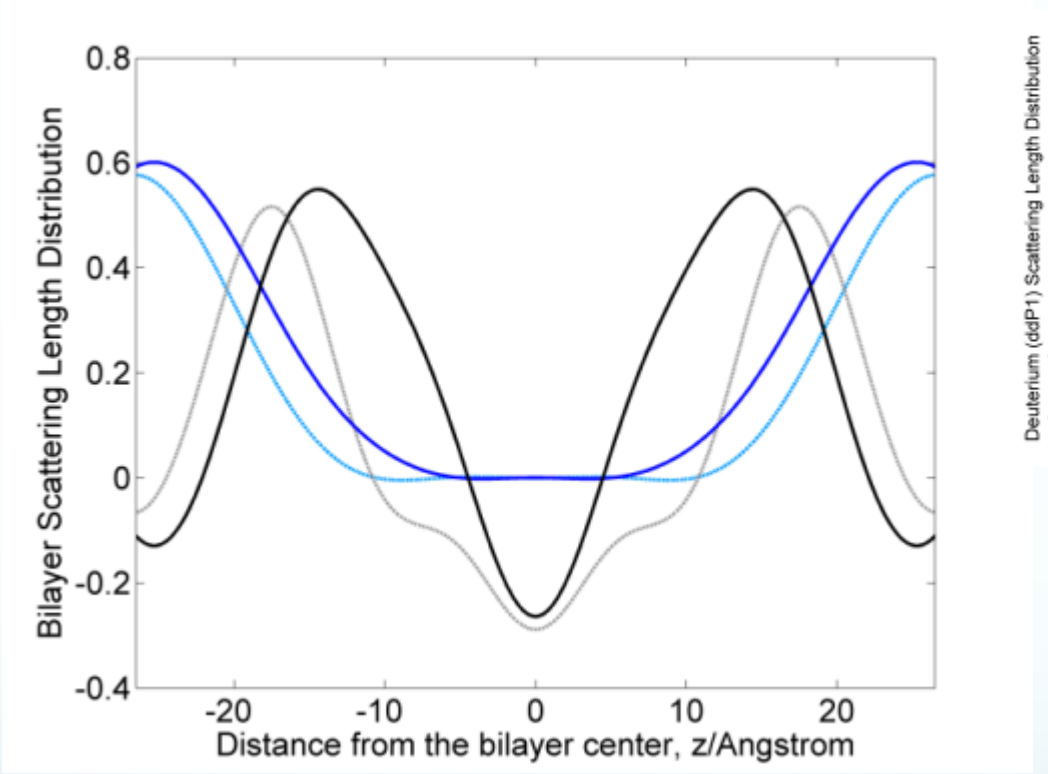
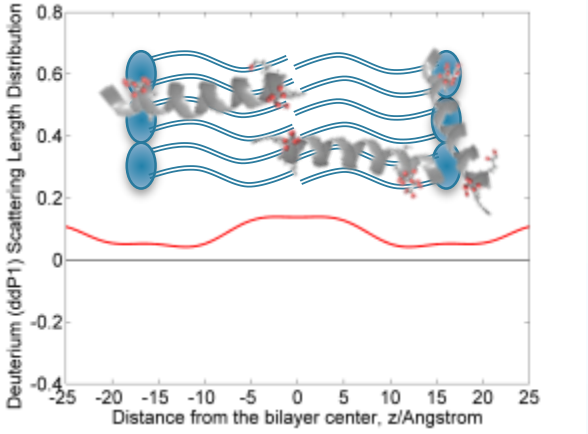
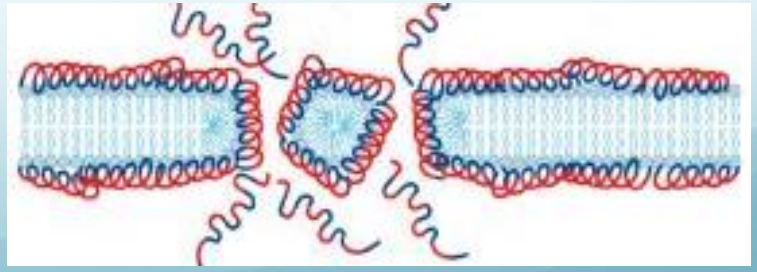


Conclusion


Bilayer Scattering Length Distribution
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-20
-10
0
10
20
Distance from the bilayer center, z/Angstrom


Deuterium (ddP1) Scattering Length Distribution
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-25
-20
-15
-10
-5
0
5
10
15
20
25
Distance from the bilayer center, z/Angstrom



Acknowledgements

- NIST SURF 2014 Directors
  - Lisa, Terrell
- IBBR
  - John Marino's group
  - Myriam Cotten (Hamilton College)
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    - Julie Borchers
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    - Cedric Gagnon
    - Yun Liu
    - Paul Butler
  - MAGIK
    - Brian Maranville
- Ella Mihailescu

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